

ASPECTS OF SEEDLING PRODUCTION OF *Clathrotropis brunnea* Amshoff, A THREATENED LEGUME TREE FROM COLOMBIAN RAINFORESTS

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Resumo

Aspectos da produção de mudas de Clathrotropis brunnea Amshoff, uma leguminosa arbórea ameaçada das florestas pluviais colombianas. A produção de mudas é crucial para auxiliar nas práticas de restauração e conservação de espécies de florestas tropicais. No presente estudo, avaliamos a produção de mudas de *Clathrotropis brunnea* Amshoff, uma leguminosa arbórea nativa das florestas tropicais da Colômbia. Os parâmetros de emergência das plântulas foram avaliados quanto a duas classes de massa das sementes (sementes pequenas < 6,0 g e sementes grandes > 6,1 g) e quanto a três graus de umidade das sementes (controle, 30% e 25%). O crescimento inicial foi avaliado em dois tamanhos de sacos de mudas (pequenos: 10 cm diâmetro x 25 cm altura, com volume de 1 700 cm³, e grandes: 15 cm x 35 cm com volume de 3 900 cm³), além do tempo de permanência no viveiro (170, 205 e 235 dias após a semeadura – DAS). Sementes grandes proporcionaram plântulas mais altas e atingiram uma porcentagem de emergência maior (93%) em relação às menores (79%). Na média, as sementes apresentaram 73% de emergência nos diferentes graus de umidade, inclusive quando dessecadas de 47% no controle (sementes frescas) a 25%. Para otimizar a produção e os custos das mudas, sementes grandes podem ser semeadas em sacos pequenos e cultivadas por no mínimo 205 DAS. O desempenho em campo das mudas produzidas ainda precisa ser testado de acordo com estas recomendações.

Palavras-chave: Fabaceae, semente recalcitrante, blackheart sapan, recipiente, viveiro

Abstract

Seedling production is crucial to assist restoration and conservation practices of tropical rainforest species. In the present study, we evaluated seedling production of *Clathrotropis brunnea* Amshoff, a legume tree native to tropical forests of Colombia. Seedling emergence parameters were assessed regarding two classes of seed mass (small seeds < 6.0 g and large seeds > 6.1 g) and tree levels of seed moisture content (control, 30% and 25%). Initial growth was evaluated in two sizes of plastic bags (small: 10 cm diameter x 25 cm height, with a volume of 1,700 cm³, and large: 15 cm x 35 cm and volume of 3,900 cm³), besides tree growth periods in the nursery (170, 205 and 235 days after sowing – DAS). Large seeds provided higher seedlings and reached a greater emergence percentage (93%) compared to the small ones (79%). On average, seeds reached 73% of emergence in the three moisture levels, including when desiccated from 47% in the control (fresh seeds) to 25%. To optimize seedling production and costs, large seeds can be sown in small bags, growing for a minimum of 205 DAS. The in-field performance of the produced seedlings remains to be tested according to these recommendations.

Keywords: Fabaceae, recalcitrant seed, blackheart sapan, container, nursery

INTRODUCTION

There is a global need for basic knowledge of seed science and seedling emergence features seeking the development of germination protocols for restoration purposes (PEDRINI; DIXON, 2020). Larger seeds usually show more reserve substances allowing for more vigorous seedlings, with a higher probability of survival under adverse conditions at a site (PEREIRA *et al.*, 2013; MACERA *et al.*, 2017). Within a species, the use of larger seeds could thus be useful to improve seedling growth performance and survival (KHURANA; SINGH, 2000).

It is known that many non-pioneer species from tropical wet forests bear large seeds, dispersed with an active metabolism and high water content (WC). Those seeds are frequently sensitive to desiccation, thus being classified as recalcitrant (MARQUES *et al.*, 2018). WC is an important factor helping to estimate seed longevity, and when this factor falls below the threshold of 25% it may provoke an intense drop in the germination capacity of recalcitrant seeds (PRITCHARD *et al.*, 2022).

Another relevant factor in the nursery is the type and size of the containers used for seedling production because they are the physical support for the substrate, air, water, and nutrients. As a result, the container size is related to biomass allocation and morphological quality of the produced seedlings (BARBOSA *et al.*, 2013; MENDONÇA *et al.*, 2020). From an economic perspective, there must be a balance between the production of well-formed seedlings and optimal container size (FREITAS *et al.*, 2018). To improve the economic efficiency of

production, the seedlings should remain for shorter growth periods in the nursery, which also depends on the container size (MENDONÇA *et al.*, 2020). Efficient production protocols might enhance seedling growth parameters, warranting a greater quality and number of seedlings that the forest nursery will be able to commercialize per year.

In this context, the sapan or blackheart sapan (*Clathrotropis brunnea* Amshoff – Fabaceae subf. Papilionoideae) is a deciduous tree species of slow growth occurring in the lowlands (from 0 m to 1,200 m altitude) of the Andean region of Colombia (AMSHOFF, 1968). The species has ecological and economic importance, its wood is used by the local communities for construction, and the sawdust presents pharmacological potential against cutaneous leishmaniasis and trypanosomiasis (TORRES *et al.*, 2020). The state of conservation of the species has not been officially evaluated by the International Union for Conservation of Nature (<https://www.iucnredlist.org/>), but within Colombia it has been recognized as endangered (CÁRDENAS; SALINAS, 2007). A previous study has shown that fresh sapan seeds are non-dormant, reaching a high emergence percentage in nursery conditions (> 70%), but short-lived in storage (PRATO *et al.*, 2021).

The present study aimed to evaluate some aspects of seedling production of *C. brunnea* by evaluating seedling emergence parameters and initial growth under nursery conditions. Hence, we assessed (a) the effects of classes of seed mass (large x small seeds) and seed moisture content (pre-desiccation treatments) on seedling emergence, and (b) the effects of container size (large x small seedling bags) and different nursery periods on seedling growth. We hypothesized that larger seeds should provide better seedling emergence and growth performance, and that a reduction in seed moisture to a lower limit of 25% before planting would still keep seed viability. In addition, we propose some contributions to optimizing the production of high-quality seedlings according to the size of the container and nursery growth periods. Such recommendations may enhance the production of high-quality seedlings for reforestation purposes, aiding in conservation and management of this threatened rainforest tree.

MATERIALS AND METHODS

Experimental site and seed collection

In October 2020, mature fruits of *C. brunnea* (dehiscent pods) were collected from the branches of seven adult trees (diameter at breast height from 28.6 to 83 cm) near the municipality of Cimitarra, department of Santander (Colombia), during two field expeditions that followed the guidelines of resolution 1466 of the *Ministerio de Ambiente y Desarrollo Sostenible de Colombia* (MinAmbiente). The collection subregion is located in the Middle Magdalena Valley (Andean region) and has a humid tropical climate (Af), according to the Köppen classification, with historical averages (1989-2019) of 27.4 °C and 2,865 mm year⁻¹ and a bimodal rainfall regime, according to the *Instituto de Hidrología, Meteorología y Estudios Ambientales*, IDEAM (<http://www.ideam.gov.co/>).

For each collection event, the pods were transported in woven mesh sacks and mixed to compose a homogeneous batch that remained in a protected environment for four days. The experiments were carried out in the facilities of *Centro de Investigación La Suiza* of the *Corporación Colombiana de Investigación Agropecuaria* (Agrosavia) located in the municipality of Rionegro, Santander, Colombia (7°22'10"N, 73°10'39"W; 550 m altitude). Seeds attacked by pests, fungi, and those malformed were discarded. Initially, the WC of freshly harvested seeds was determined using three replicates of five seeds each. They were cut down the middle and dried in an oven at 105 °C for 24 hours, then weighed on an analytical scale (0.01 g precision) and the results were expressed in percentages.

Seedling emergence tests: classes of seed mass and seed moisture content

Seed fresh mass was characterized using a sample of 200 individual seeds measured on an analytical scale (0.01 g). Seed dimensions were measured using the same sample, with the aid of a digital caliper (0.01 mm), considering seed length (longest axis from seed base to the apex) and seed width (in the median portion of the seed axis). A histogram with nine classes of seed mass was obtained through the Sturges formula (Supplementary material, Figure S1). Then, two main classes of seed mass were selected by dividing seeds around the mean of the size distribution. Hence, small seeds (ss) were classified as those smaller than 6.0 g, while large seeds (LS) were classified as those larger than 6.1 g. To evaluate the effect of classes of seed size on seedling emergence, a completely randomized block design with two treatments (i.e., small seeds vs. large seeds) was adopted in the nursery, using four replicates of 25 seeds in each treatment.

In a separate experiment, the effect of seed moisture content on seedling emergence was evaluated. To do so, the seeds were subjected to a rapid drying process with silica gel prior to sowing until they reached two predefined moisture contents: 30% and 25%. The seeds were uniformly distributed in plastic trays containing activated silica gel (1 to 3 mm grains), which was periodically replaced when it lost the blue color code. The process was conducted under laboratory conditions (20 °C) and a ratio of 3:1 (mass/mass) was used for silica gel

in proportion to seed mass. The seed batch was weighed every two to four hours until the predefined water content was reached, as described by Hong and Ellis (1996). The final WC was tested by oven drying seeds at 105 °C for 24 hours, as described above. There was a control treatment consisting of fresh seeds without desiccation (initial WC ~ 47%). A completely randomized block design with three treatments (control, 30% and 25%) was thus adopted to evaluate seedling emergence in the nursery, each one with three replicates of 25 seeds due to restraints in seed availability.

The seedling emergence tests were performed in an agricultural nursery with plastic cover (Agrofrío X, 65% shade) and automated micro-sprinkler irrigation. Seeds were sowed with hilum downwards in conical tubes (8 cm diameter x 24 cm height; volume of 700 cm³) containing river sand, which was maintained at field capacity by direct observation. There was no pre-germinative pretreatment, given *C. brunnea* seeds are non-dormant (PRATO *et al.* 2021). The number of emerged seedlings was registered when the epicotyl was visible above the substrate surface forming normal seedlings. The relative average temperature and humidity within the nursery were 25.1 ± 4.8 °C and 91.2 ± 13%, respectively. Following the Ranal and Santana (2006) formulas, seedling emergence percentage (SE) and mean emergence time (MET) were calculated. At 60 days after sowing (DAS) the end of the emergence process was verified, and the stem diameter (SD) was measured in all normal seedlings with a digital caliper (0.01 mm). Seedling height (H) was measured with a metric tape (0.1 cm), from the stem base to the terminal bud, the number of leaves was counted, and the robustness index (RI) was calculated as the H / SD ratio.

Seedling growth tests: container size and nursery growth period

Seedlings emerged from each class of seed mass (small seeds = ss, and large seeds = LS) were transplanted at 65 days to plastic bags (containers) of two different sizes: small bags (sb) = 1,700 cm³ (10 cm diameter x 25 cm height) and large bags (LB) = 3,900 cm³ (15 cm x 35 cm). A completely randomized block design was adopted in a factorial arrangement of 2 x 2 (two container sizes x two seed masses) with three repetitions of eight seedlings for small bags and six seedlings for large bags. Seedling growth was evaluated at 90 and 120 DAS, and subsequently every 15 days until the end of the evaluation at 235 DAS. Stem diameter, height, number of leaves, and robustness index were measured in each evaluation period. In addition, destructive variables of plant dry mass were determined by the end of the tests at 235 DAS (see below).

To evaluate seedling growth periods in the nursery, the seedlings resulting from the seed moisture content emergence test were grouped without distinction of treatment and transplanted at 60 DAS in plastic bags of 1,700 cm³ (small bags). A completely randomized block design was adopted with three treatments of growth period in the nursery (i.e., 175, 205, and 235 DAS) and three replicates of eight seedlings per treatment.

Seedling growth was evaluated under nursery conditions for both tests, covered by black polyshade at 50% and automated micro-sprinkler irrigation. The average temperature was 26.1 ± 5.6 °C and the relative humidity of 86.6 ± 18%. As described above, the stem diameter, height, number of leaves and robustness index were determined in each evaluation period for both tests. For the container size x seed mass experiment, the destructive variables were determined only at 235 DAS, as mentioned, while for the nursery growth periods they were measured at the three observed times: 175, 205, and 235 DAS. Hence, the destructive variables consisted of shoot dry mass (SDM) and root dry mass (RDM), measured by drying the plant material in an oven (65 °C) until reaching constant weight, determined on an analytical scale. With these values, the Dickson's quality index (DQI) was determined, according to the formula:

$$DQI = (SDM + RDM) / ((H / SD) + (SDM / RDM))$$

Where H = height (cm), SD = stem diameter (mm), SDM = shoot dry mass (g), and RDM = root dry mass (g).

The used substrate (sieve = 4 mm) was based on a local formulation of 60% soil + 20% river sand + 20% commercial compost (obtained from composting poultry manure, sugar cane dust and sawdust powder). A sample of the substrate was sent to the *Laboratorio de Suelos* of the *Universidad Nacional de Colombia - Medellín* for physical and chemical properties characterization. The calculation of the bulk density (Db) followed the methodology described by MAPA (2007). The results showed a Db = 1.26 g cm⁻³, organic matter = 34 g kg⁻¹ (modified Walkley and Black method), pH = 8.2 (pH meter and ratio soil:water of 1:2.5), available P = 432 mg kg⁻¹ (Bray II method), Ca⁺², Mg⁺², and K⁺ had values of 8.22, 3.63, and 4.03 cmol_c kg⁻¹ exchangeable (atomic emission spectrometer using a 1 N KCl extraction), respectively.

Data analysis

Normality and homoscedasticity of variance of the data were checked by means of Shapiro-Wilk and Levene tests, respectively. One-way analysis of variance was performed to evaluate statistical differences between treatments in the seedling emergence experiments (classes of seed mass and seed moisture contents) and the

seedling growth periods in the nursery. A two-way analysis of variance was performed to evaluate the container size x seed mass experiment. The analyses were followed by the T-test of student or post-hoc Tukey test ($p < 0.05$). The variables of seedling height, stem diameter, robustness index and number of leaves in the seedling growth test (experiment: container sizes x classes of seed mass) were subjected to polynomial regression analysis. The model with the highest coefficient of determination (R^2) and significance of the parameters estimated by the F-test was selected. A Pearson correlation analysis was applied between all destructive and non-destructive variables by pooling the final results obtained from the seedling growth experiments. The analyses were performed in the statistical program S.A.S 9.3.

RESULTS

Seedling emergence tests

Seedling emergence percentage was significantly higher for the large seeds, reaching 93%, against 79% for the small seeds (Table 1). Larger seeds also displayed faster MET values, lasting around three days less (33.9 days) in comparison to the small seeds (36.6 days). By the end of the experiment, the large seeds showed a significant increase in seedling height (24.7 cm), number of leaves per seedling (2.2) and greater stem diameter (4.76 mm), that is, an average increase of 26% in relation to the seedlings that emerged from the small seeds (Table 1). In contrast, robustness index maintained similar values between seedlings from large or small seeds (Supplementary material Table S1).

In relation to seed moisture contents, the emergence percentage was similar irrespective of WC desiccation, varying from 68% to 73% at WC of 30% and 25%, respectively. Seedling emergence reached 79% for fresh seeds in the control (WC = 47%; Table 2). Likewise, the treatments had no significant effect on MET values (means = 35.7 days; Table 2) and also no effect on the other seedling emergence parameters (seedling height = 20.3 cm, stem diameter = 3.97 mm, number of leaves = 1.1 and robustness index = 5.1 cm mm^{-1} ; data not shown) (Supplementary material Table S2)

Table 1. Values (means \pm standard error) of seedling emergence (SE, %), mean emergence time (MET, days), height (H), stem diameter (SD), number of leaves (L) and robustness index (RI) of seedlings of *Clathrotropis brunnea*, according to two classes of seed mass at 60 days after sowing.

Tabela 1. Valores (média \pm erro padrão) de emergência de plântulas (SE, %), tempo médio de emergência (MET, dias), altura (H), diâmetro do colo (SD), número de folhas (L) e índice de robustez (RI) de plântulas de *Clathrotropis brunnea*, de acordo com duas classes de massa da semente aos 60 dias após a semeadura.

Seed mass	SE (%)	MET (days)	H (cm) *	SD (mm)	L (unit)	RI (cm mm^{-1})
Small	79.0 \pm 2.5 b	36.6 \pm 0.3 a	20.7 \pm 0.8 b	4.18 \pm 0.1 b	1.5 \pm 0.1 b	5.00 \pm 0.3 ns
Large	93.0 \pm 1.0 a	33.9 \pm 0.5 b	24.7 \pm 0.0 a	4.76 \pm 0.1 a	2.2 \pm 0.2 a	5.20 \pm 0.1
Means	86.0	35.3	22.7	4.47	1.9	5.09

Means followed by the same letter in the columns do not differ from each other by test t – student ($p < 0.05$). * data transformed by log n. ns= not significant.

Table 2. Values (means \pm standard error) of seedling emergence (SE, %) and mean emergence time (MET, days) of seedlings of *Clathrotropis brunnea*, according to tree levels of seed moisture content at 60 days after sowing.

Tabela 2. Valores (média \pm erro padrão) de emergência de plântulas (SE, %) e tempo médio de emergência (MET, dias) de plântulas de *Clathrotropis brunnea*, de acordo com tres níveis de umidade das sementes, aos 60 dias após a semeadura.

WC (%)	Seedling emergence (%)	Mean emergence time (days)
Control (47%)	78.7 \pm 6.1 ns	34.1 \pm 0.9 ns
30	68.0 \pm 4.0	37.6 \pm 2.2
25	73.3 \pm 2.3	35.5 \pm 0.7
Means	73.3	35.7

Different letters in the columns indicate significant differences between each other according to Tukey test ($p < 0.05$). ns = not significant.

Seedling growth tests

Regarding the container size x seed mass experiment (see Figure 1A), there was a slow increase in seedling height over time for all treatments, adjusting to the linear models (values of $R^2 > 0.80$; Figure 2A).

Container size had no influence on seedling height, which increased by 16% at the end of the experiment (235 DAS) either for small or large bags in relation to the initial values (Figure 2A). There was a slight difference between the two classes of seed mass, with the large seeds showing higher seeds by around 20% when compared to the small ones (large seeds = 34.4 cm, small seeds = 29.2 cm at 235 DAS). The number of leaves increased from two to four times in the treatments with respect to the initial values, reaching around 10 leaves per seedling in the combination of large seeds in small bags (Figure 2B). Stem diameter also increased through time (~4 mm initial to 8 mm at 235 DAS) and the difference between bag sizes was less (6% to 8%) than between seed masses (12% to 14%; Figure 2C). At 235 DAS, the robustness index was reduced by half of its initial values, independently of treatments, decreasing from an average value of 6.40 cm mm⁻¹ to 3.65 cm mm⁻¹ (Figure 2D, Supplementary material Table S3).

Biomass accumulation of shoot dry mass (SDM) and root dry mass (RDM) was 44% and 32% greater, respectively, for the seedlings originating from large seeds than in the small seeds, without differences between bag sizes, at the end of the experiment (Table 3). The treatments had no significant effect on the SDM/RDM ratio (means = 1.94), whereas the Dickson's quality index tended to be mildly higher for large seeds in small bags (DQI = 1.65; Table 3, Supplementary material Table S3).

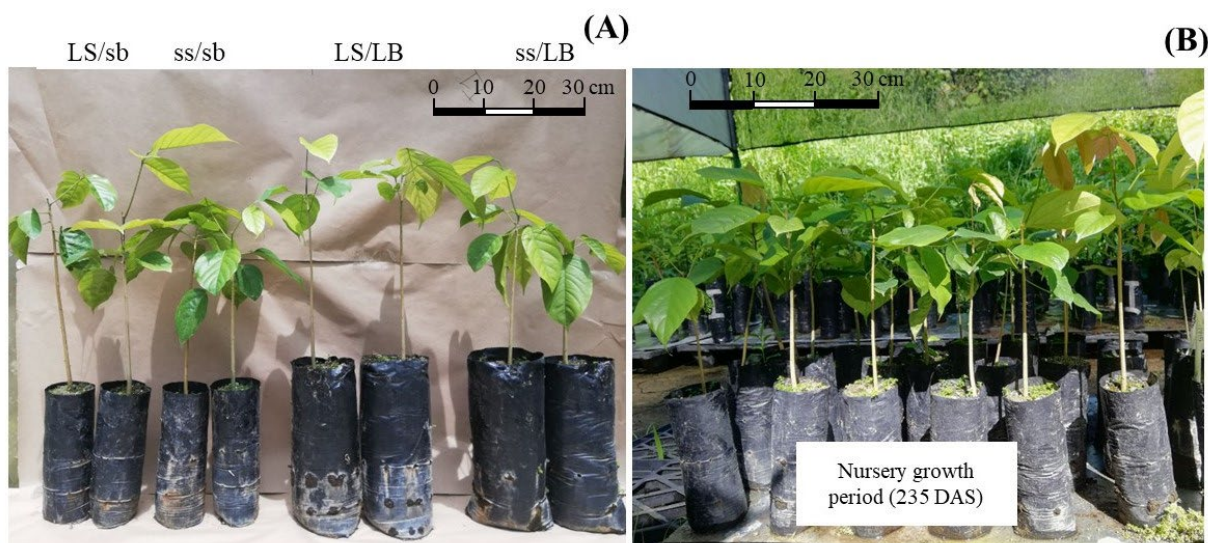


Figure 1. Seedlings of *Clathrotropis brunnea* at 235 days after sowing (DAS) according to A) container sizes and classes of seed mass and B) nursery growth periods. LS/sb= large seed x small bag; ss/sb = small seed x small bag; LS/LB = large seed x large bag; ss/LB = small seed x large bag.

Figura 1. A) Mudanças de *Clathrotropis brunnea* aos 235 dias após a semeadura (DAS) de acordo com A) tamanho do recipiente e classes de massa da semente e B) tempo de permanência em viveiro. LS/sb = semente grande x recipiente pequeno; ss/sb = semente pequena x recipiente pequeno; LS/LB = semente grande x recipiente grande; ss/LB = semente pequena x recipiente grande.

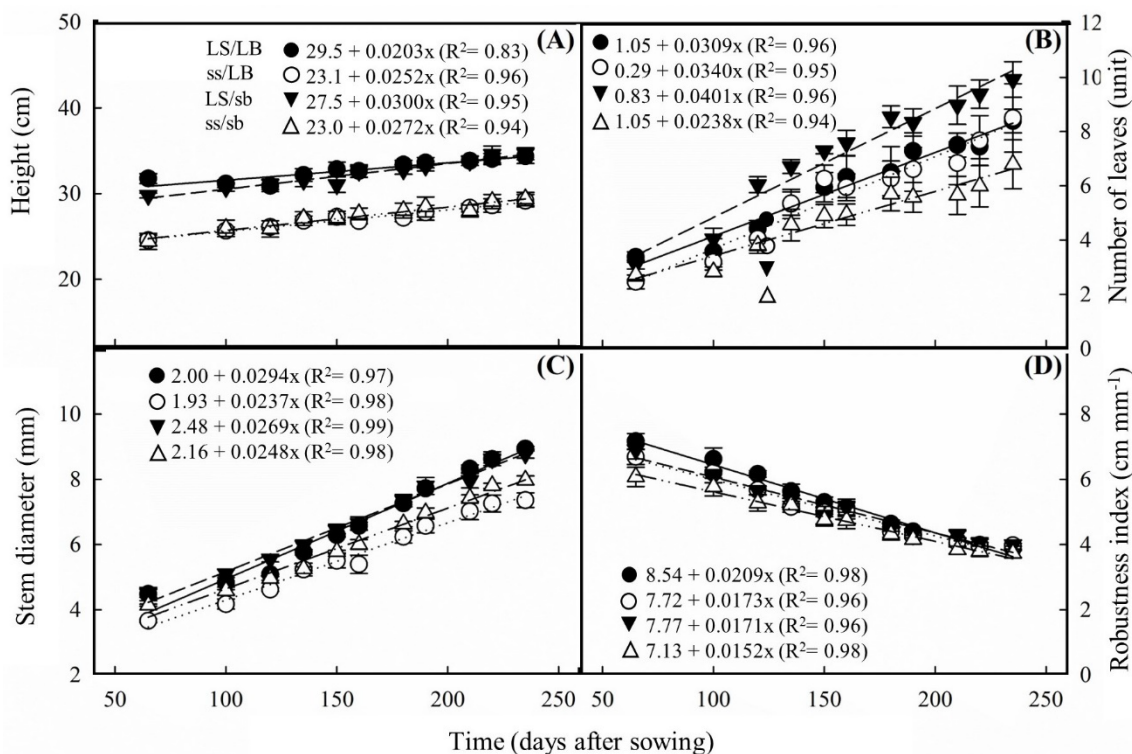


Figure 2. A) seedling height, B) number of leaves, C) stem diameter and D) robustness index in seedlings of *Clathrotropis brunnea* according to container size (plastic bag) and seed mass, growing until 235 days after sowing. LS/LB = large seed x large bag; ss/LB = small seed x large bag; LS/sb = large seed x small bag; ss/sb = small seed x small bag. Data points represent means of tree replicates and bars represent the standard error.

Figura 2. A) altura das plântulas, B) número de folhas, C) diâmetro do caule e D) índice de robustez em mudas de *Clathrotropis brunnea* de acordo com o tamanho do recipiente (saco plástico) e a massa da semente, cultivadas até 235 dias após a semeadura. LS/LB = semente grande x recipiente grande; ss/LB = semente pequena x recipiente grande; LS/sb = semente grande x recipiente pequeno; ss/sb = semente pequena x recipiente pequeno. Os pontos de dados representam a médias de três réplicas e as barras representam o erro padrão.

Table 3. Values (means \pm standard error) of shoot dry mass (SDM), root dry mass (RDM), SDM/RDM ratio and Dickson's quality index (DQI) of seedling of *Clathrotropis brunnea*, according to container size and seed mass, at 235 days after sowing.

Tabela 3. Valores (média \pm erro padrão) de massa seca da parte aérea (SDM), massa seca da raiz (RDM), relação SDM/RDM e índice de qualidade de Dickson (DQI) de mudas de *Clathrotropis brunnea*, de acordo o tamanho dos recipientes e a massa da semente, aos 235 dias após a semeadura.

Size		SDM	RDM	SDM/RDM ratio	DQI
Seed / Bag		(g seedling ⁻¹)			
Small	Small	5.47 \pm 0.2 b	2.92 \pm 0.1 b	1.88 \pm 0.0 ns	1.244 \pm 0.04 b
	Large	5.35 \pm 0.5 b	2.93 \pm 0.1 b	1.82 \pm 0.1	1.176 \pm 0.09 b
Large	Small	8.19 \pm 0.3 a	4.23 \pm 0.2 a	1.94 \pm 0.1	1.650 \pm 0.03 a
	Large	7.40 \pm 0.4 a	3.47 \pm 0.3 ab	2.14 \pm 0.1	1.495 \pm 0.08 ab
Means		6.60	3.39	1.94	1.391

Different letters in the columns indicate significant differences between each other according to the test Tukey ($p < 0.05$). ns = not significant.

Seedling growth parameters mostly increased with the observed nursery growth periods (Fig. 1B), except for seedling height and robustness index, which had no difference over time (Table 4). The values of stem diameter (means = 6.96 mm), DQI (means = 1.148) and SDM (means = 4.76 g seedling⁻¹) showed similar values at 205 and

235 DAS, with a significant increase when compared to 175 DAS (Table 4). The number of leaves and SDM/RDM ratio only showed a significant difference at 235 DAS, with a 20% increase and a 21% decrease in their initial values, respectively. RDM showed a significant increase at all observed periods (reaching 2.58 g seedling⁻¹ at 235 DAS; Table 4, Supplementary material Table S4).

There was a strong linear correlation (positive or negative) between most of the seedling growth variables (Table 5). Based on the highest relative coefficient of determination values, the stem diameter stood out among the other nondestructive variables as the best estimator of the destructive variables SDM, RDM, and DQI ($R^2 > 0.92$). The SDM/RDM ratio and robustness index had the lowest degree of relationship with the other parameters. The positive correlation between SDM with RDM, and these two with the DQI showed up to be very strong ($R^2 > 0.95$; Table 5).

Table 4. Values (means \pm standard error) of seedling height, stem diameter, number of leaves, robustness index, shoot dry mass (SDM), root dry mass (RDM), SDM/RDM ratio and Dickson's quality index (DQI) of seedling of *Clathrotropis brunnea*, according to three nursery growth periods.

Tabela 4. Valores (médias \pm erro padrão) de altura, diâmetro do caule, número de folhas, índice de robustez, massa seca da parte aérea (SDM), massa seca da raiz (RDM), relação SDM/RDM e índice de qualidade de Dickson (DQI) de mudas de *Clathrotropis brunnea*, de acordo com três períodos de permanência em viveiro.

Period (DAS)*	Height (cm)	Stem diameter (mm)	Number of leaves (unit)	Robustness index (cm mm ⁻¹)
175	27.1 \pm 0.6 ns	5.79 \pm 0.1 b	5.2 \pm 0.0 b	4.77 \pm 0.1 ns
205	27.5 \pm 1.3	6.71 \pm 0.2 a	6.1 \pm 0.3 ab	4.16 \pm 0.3
235	29.2 \pm 0.5	7.21 \pm 0.3 a	7.9 \pm 0.6 a	4.65 \pm 0.1
Means	27.9	6.57	6.4	4.34

Period (DAS)*	SDM	RDM	SDM / RDM ratio	DQI
	(g seedling ⁻¹)			
175	2.91 \pm 0.2 b	1.16 \pm 0.0 c	2.52 \pm 0.1 a	0.565 \pm 0.0 b
205	4.36 \pm 0.1 a	1.99 \pm 0.1 b	2.19 \pm 0.1 ab	1.015 \pm 0.1 a
235	5.15 \pm 0.3 a	2.58 \pm 0.1 a	2.00 \pm 0.0 b	1.281 \pm 0.1 a
Means	4.14	1.91	2.23	0.954

Different letters in the columns indicate significant differences between each other according to the test Tukey ($p < 0.05$). ns = not significant.
* DAS = days after sowing.

Table 5. Matrix of Pearson's correlations coefficients for nondestructive and destructive variables of seedling growth of *Clathrotropis brunnea*: height (H), stem diameter (SD), number of leaves (L), robustness index (RI), shoot dry mass (SDM), root dry mass (RDM), DSM/RDM ratio and Dickson's quality index (DQI).

Tabela 5. Matriz de coeficientes de correlações de Pearson para as variáveis não destrutivas e destrutivas do crescimento de plântulas de *Clathrotropis brunnea*: altura (H), diâmetro do caule (SD), número de folhas (L), índice de robustez (RI), massa da parte aérea seca (SDM), seca massa de raiz (RDM), relação SDM/RDM e índice de qualidade de Dickson (DQI).

	H	SD	RI	L	SDM	RDM	SDM/ RDM ratio	DQI
H	1							
SD	0.84**	1						
RI	-0.31	-0.72**	1					
L	0.75**	0.69**	-0.39	1				
SDM	0.91**	0.95**	-0.59**	0.81**	1			
RDM	0.82**	0.92**	-0.65**	0.83**	0.96**	1		
SDM/ RDM ratio	-0.19	-0.50*	0.68**	-0.48*	-0.44*	-0.65**	1	
DQI	0.77**	0.94**	-0.73**	0.79**	0.95**	0.96**	-0.60**	1

* and ** are significant at 5% and 1% level, respectively. In bold type only very strong correlations, assuming an absolute determination coefficient of $R^2 > 0.9$.

DISCUSSION

Our data shows that seedling emergence is affected by classes of seed mass, reaching 93% among the large seeds and 79% for the small ones. Furthermore, seedling emergence from large seeds was slightly faster (Table 1). In a global sense, species with larger seeds tend to occur in the tropics (MOLES *et al.*, 2007), frequently without presenting dormancy, especially in humid areas (WYSE; DICKIE, 2018). Although large seeds may germinate more quickly, reducing the risk of predation, this relationship has not been found at a community level among tropical rainforests (NORDEN *et al.*, 2009). Nevertheless, seed size was important to explain emergence and growth parameters of the sapan seedlings.

Despite size variation, the sapan seeds are generally large (means of mass = 9.1 g, length = 4.4 cm and width = 3.0 cm; PRATO *et al.*, 2021). For 78 families of trees native to the Amazon, it was found that large seeds (> 2 cm) are more associated with warmer and aseasonal tropical rainforests than small seeds (< 0.99 cm) (MALHADO *et al.*, 2015). Such conditions which are also characteristics of the region where our study seeds were collected. Although sapan seeds can be short-lived in storage (PRATO *et al.*, 2021), seeds dried up to 25% WC maintained similar emergence parameters, which indicates that sapan seedlings can be produced successfully at least up to this limit of water loss. With continuous desiccation, however, the germination and survival of sapan seeds tends to zero around 13% of WC (PRATO *et al.*, 2021).

Seedling emergence and growth parameters evaluated under nursery conditions can predict plant performance after planting, i.e., survival and initial growth (BUDIMAN *et al.*, 2015; MENDONÇA *et al.*, 2020). In the seedling growth experiments, seedling height showed an increase of around 5 cm (0.55 cm month⁻¹) related to seed mass and only a slight difference over time, suggesting a slow growth of the species. In addition to seedling height, other nondestructive parameters of easy measurement were important to monitoring seedling growth, such as stem diameter, with a strong increase over time that can be related to in-field seedling survival (AVELINO *et al.*, 2021). Moreover, stem diameter was the seedling quality parameter most strongly correlated with the others, suggesting it has great reliability. Likewise, the number of leaves is considered a proxy for the total leaf area synthesizing photoassimilates, as shown for *Enterolobium contortisiliquum*, another tropical legume tree (FREITAS *et al.*, 2018). Similar results were found for other native species of rainforests in Asia and Brazil (BUDIMAN *et al.*, 2015; AVELINO *et al.*, 2021).

Other forest species reach a seedling height from 20 to 30 cm and a stem diameter of around four mm (DAVIDE; BOTELHO, 2015), which is similar to the values we have found (29.2 to 34.6 cm in height and 5.79 to 8.91 mm SD) between 175 to 235 DAS in the seedling growth tests. Even by the end of the emergence tests at 60 to 65 DAS, the observed values were very close (19.8 to 24.7 cm and 3.90 to 4.76 mm). These values concur with reports by Prato *et al.* (2021) for the sapan seedling emergence at 60 DAS, corroborating the efficiency of the adopted propagation techniques from seeds of the study species.

Container sizes did not affect seedling growth, hence there was no restriction for the development of the root system in the small bags. Because the small bags provide similar seedling growth, they should be used to optimize the costs of production. Although container sizes seem to influence seedling production of tropical species (BARBOSA *et al.*, 2013), differences in seedling growth may tend to disappear over time in the field, as shown for *Psidium cauliflora* (Myrtaceae) from the Atlantic Forest (MENDONÇA *et al.*, 2020). Seedling biomass accumulation was higher for the large seeds, reinforcing that seedlings from large seeds are more vigorous and of higher quality. Seed size has shown to be a good indicator of seedling establishment and survival under field conditions for several tropical legume trees (PEREIRA *et al.*, 2013; MACERA *et al.*, 2017). Future studies should establish quality parameters that allow for monitoring field establishment of the sapan seedlings under different conditions.

Optimal values for the SDM/RDM ratio are situated between two and three, providing better resistance of seedlings to stressful conditions, reflecting the balance between photosynthetic capacity (shoot) with the anchoring and absorption of nutrients and water (root) (GROSSNICKLE; MACDONALD, 2018). Thus, the measured ratio in the sapan seedlings is considered acceptable in all treatments independent of container size, seed mass, and growth period. The DQI, which gathers destructive and nondestructive variables in the same index, indicates that the greater the value, the greater the quality of the seedling. The best DQI values were observed for large seeds irrespective of container size (≥ 1.5), and from 205 DAS onwards (> 1.0) when seed masses were pooled. Probably a faster growth in biomass of the seedlings from large seeds may contribute to reducing the period of permanence in the nursery. Additionally, the final value of the robustness index between 175 and 235 DAS (4.17 to 4.77 cm mm⁻¹) or between both container sizes and classes of seed mass (3.56 to 3.63 cm mm⁻¹) evidenced a similar growth pattern in most treatments.

Given our findings, there is no need for using large containers for seedling production, which increases substrate volume by 1.2 times and thus the cost per bag. The present study confirmed that under the evaluated nursery conditions, there is no significant benefit for the sapan seedlings being kept up to 235 DAS for most growth

parameters when seed masses were pooled. Nevertheless, an additional growing period of a month after 175 DAS leads to an improvement of SDM, RDM, and DQI between 50% and 80%.

CONCLUSIONS

- Although recalcitrant, *C. brunnea* seeds may keep the emergence capacity and vigor of seedlings obtained from pre-desiccated seeds until 25% of seed moisture before sowing.
- We recommend sowing from large seed mass (> 6.1 g), utilizing bags with a volume of 1,700 cm³ and dimensions of 10 cm diameter x 25 cm height, and growing up to 205 days in the nursery to optimize the production of sapan seedlings for its use in reforestation projects.

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